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Characterization and functional properties of proteins isolated from flaxseed cake and sesame cake

 MUHAMMAD ELSAYED ELSORADY*

Oils and Fats Research Department, Food Technology Research Institute, Agricultural Research Center, Giza, Egypt

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ABSTRACT

Flaxseed protein isolate (FPI) and sesame protein isolate (SPI) were extracted from flaxseed and sesame cake as by-products, and their functional properties (water holding and fat absorption capacities, bulk density, least gelling concentration, solubility, and emulsifying properties) were determined. Bulk density of the SPI (0.162 g/ml) was lower than that of the FPI (0.175 g/ml). The water absorption capacity of the FPI (305.66%) was higher than that of the SPI (288.93%). The oil absorption capacity and least gelling capacity of the FPI and SPI were 127.48, 3.6, 134.39, and 5%, respectively. The least solubility occurred at pH 4.0 and it was 24.54, 9.56% for FPI and SPI, respectively. Levels of pH and salt concentrations were used as dependent variables for the characterization of emulsifying capacity, activity, and emulsion stability, as well as foaming capacity and foam stability. The addition of NaCl at concentrations up to 1.0 M improved these characteristics. The SPI and FPI had a minimum emulsion capacity (74.54 and 100.20 ml oil/g protein, respectively) and a minimum foam capacity (14.25 and 17.35 %, respectively) at pH 4. The FPI and SPI were found to be highly soluble at acidic and alkaline pH and their emulsifying and foaming properties were high. Moreover, their bulk density, water absorption, and fat absorption capacities and least gelling capacity properties were good. Therefore, the FPI and SPI can be used in food formulation systems.

Introduction

Flaxseed (*Linum usitatissimum*) is an annual plant of the Linaceae family. It contains seeds known as flaxseeds or linseeds. Flaxseed is one of the oldest crops known to man and it is cultivated for fibre and oil. It was used for medical purposes in ancient Egypt and Greece, and also as an energy source (Rubilar et al., 2010). The annual Egyptian production of flaxseed during 2017 was 5000 tons (FAO, 2017). Flaxseed meal and cakes, by-products of oil extraction, have had limited use as protein supplements in livestock rations. In recent years, flaxseed popularity in the food and feed markets has increased because of the reported health benefits and disease preventive properties attributed to flaxseed components. However, flaxseed protein, one of the major components of flaxseed meal,

remains underutilized, as procedures for its extraction are complicated and not commercially viable (Oomah et al., 1994). Most of the protein is concentrated in flaxseed meal, a by-product of the flaxseed-crushing industry (Oomah, 2001).

Because of its high content of lipids, sesame (*Sesamum indicum* L., *Pedaliaceae*) is one of the most important oilseed crops in the world. It is not only a source of edible oil, but also widely used in baked goods and confectionery products. In Egypt, the major part of the imported sesame is essentially transformed to Halaweh. This food product is obtained after mixing the white tehneh (white sesame seed dehulled, roasted, and ground), saponin (*Saponaria officinalis*), and nougat (heat-treated sucrose). Sesame cake is a by-product of the oil industry which could be recovered and used as a value-added product. However, in some sesame processing countries, this by-product is

*Corresponding author E-mail: muhammadelorady@yahoo.com

generally discarded or used as animal feed (Abdelazim et al., 2013).

The defatted sesame cake is a good source of protein, 50% approximately, and has a high content of methionine and tryptophan that distinguishes sesame from other oil seeds. This cake is used as a protein source or as an ingredient in the food industry. In order for plant proteins to be useful and successful in food application, they should ideally possess several desirable characteristics, referred to as functional properties, as well as providing essential amino acids (Bukya and Vijayakumar, 2013). Protein obtained from sesame cake presents a potential nutritional alternative for human consumption or for use as a raw material in the food industry as a functional or nutritional ingredient in a wide variety of food products (Escamilla-Silva et al., 2003).

Functional properties of protein are important in food processing and food formulation. Some of these properties are solubility, water and oil absorption capacity, foaming capacity and stability, gelation, bulk density, and viscosity (Onsaard, 2012). The sensory characteristics of foods are complemented by the functional properties, which play important roles in the physical behaviour of food or its ingredients during preparation, processing, and storage. Many food industries intensify the desirable functional properties in order to improve them to meet their requirements (Kaur et al., 2017).

Therefore, the objectives of this study are to utilize byproducts and to investigate the characterization and functional properties of protein isolates from flaxseed cake and sesame cake.

Materials and methods

Materials

Flaxseed and sesame cakes were obtained as byproducts of the oil industry. The chemicals and reagents used were of analytical and food grade quality obtained from Sigma Chemical Co. (ST. Louis, US) and El-Gomhoria Co. for Pharmaceutical, Cairo, Egypt.

Methods

Proximate analysis of flaxseed and sesame cakes

Chemical compositions of flaxseed and sesame cakes were determined in triplicate according to AOAC (2005). Carbohydrate content was calculated by difference.

Mucilage removal

In order to remove mucilage, flaxseed and sesame cakes were mixed with a 0.5 M NaHCO₃ solution at a 1:8 ratio (w/v) and stirred at 500 rpm for 18 h at room temperature (Marambe et al., 2008). Seed cakes were recovered by filtration, manually rubbed against an aluminium wire mesh, and washed thoroughly with Milli-Q™ water. The extraction and washing procedures were repeated twice.

Isolation of the flaxseed protein isolate (FPI) and sesame protein isolate (SPI)

Defatting of flaxseed and sesame cakes

Defatted flaxseed and sesame cakes were defatted with hexane (1:3, w/v) at ambient temperature (21±2 °C) for 24 h with the renewal of hexane every 6 h, yielding defatted flaxseed and sesame cakes.

Flaxseed protein isolate (FPI) and sesame protein isolate (SPI) were extracted from defatted flaxseed cake and defatted sesame cake, respectively, according to the methodology described by Silva et al. (2013).

Physical and functional properties of flaxseed protein isolates (FPI) and sesame protein isolates (SPI)

Bulk density was determined using the method described by Monteiro and Prakash (1994), and was expressed as gram per millilitre (g/ml). Water absorption capacity (WAC) was determined using the method described by Rodriguez-Ambriz et al. (2005). Oil absorption capacity (OAC) was determined using the method described by Lin and Zayas (1987). Least gelling concentration was determined using the method of Sathe and Salunkhe (1981). The solubility of the FPI and SPI were determined as a function of pH, using the method of Morr et al. (1985).

Emulsion capacity was determined according to the procedure of Beuchat et al. (1975) and was expressed as millilitres of oil emulsified per g of protein. Emulsifying activity index (EAI) and Emulsion stability index were determined according to the method of Gbadamosi et al. (2012). Foam capacity and stability were evaluated according to Chavan et al. (2001) and Ogunwolu et al. (2009). These functional properties were determined as a function of pH and NaCl concentration.

Statistical analysis

Statistical analyses were conducted using the SPSS program version 16.0.

Results and discussion

Proximate analysis of flaxseed and sesame cakes

Table (1) shows the chemical composition of flaxseed and sesame cakes. Data revealed that flaxseed cake had 9.27% moisture. This agreed with Oomah et al. (1994) and Silva et al. (2013). Sesame cake had a higher content of moisture, protein, and ash than flaxseed cake. This indicates that byproducts (flaxseed and sesame cakes) could be used as a source of protein. The protein content in sesame cake was lower than 59% reported by Achouri and Boye (2013). The protein content in flaxseed cake was 38.41%, which agreed with that reported by Oomah et al. (1994).

Table 1. Proximate analysis of flaxseed and sesame cakes

Components	Flaxseed cake	Sesame cake
Moisture	9.27±0.21 ^a	10.19±0.14 ^b
Fat	12.88±0.13 ^a	10.41±0.06 ^b
Protein	38.41±0.64 ^a	40.98±0.55 ^b
Ash	5.03±0.04 ^a	6.10±0.05 ^b
Carbohydrates	34.41±0.35 ^a	32.31±0.53 ^b

*a-b different superscripts indicate significant differences (p<0.05)

Physical and functional properties of the flaxseed protein isolate (FPI) and sesame protein isolate (SPI)

Physical properties

The bulk density of the FPI and SPI were illustrated in Table (2), where the bulk density of the SPI (0.162 g/ml) was lower than that of the FPI (0.175 g/ml). This was in agreement with the report of Kanu et al. (2007) for sesame protein isolate (0.169 g/ml). Krause et al. (2002) and Kanu et al. (2007) attributed the low bulk density of the protein isolate to the fact that since protein isolate is rich in protein, there will be little or low amounts of carbohydrates that usually increase the bulk density of most food products. Bulk density of a material is important in relation to its packaging (Akubor, 2007). An increase in bulk density is desirable in that it offers greater packaging advantage, as a greater quantity may be packed within a constant volume (Fagbemi, 1999).

Table 2. Physical and functional properties of the FPI and SPI

Sample	Bulk density (g/ml)	Water absorption capacity (%)	Oil absorption capacity (%)	Least gelling capacity (%)
FPI	0.175±0.00 ^a	305.66±2.40 ^a	127.48±0.91 ^a	3.60±0.0 ^{0a}
SPI	0.162±0.00 ^b	288.93±1.01 ^b	134.39±1.09 ^b	5.00±0.0 ^{0b}

*a-b different superscripts indicate significant differences (p<0.05)

Functional properties

Water absorption capacity (WAC) is an important parameter affecting the viscosity of food products. Oil absorption capacity (OAC) is an indicator rate of protein binds to fat in food formulations (Singh et al., 2005). The WAC of the FPI (305.66%) was higher than that of the SPI (288.93%). This was lower than reported by Kanu et al. (2007) for sesame protein isolate (302%). Interactions of water with proteins are very important in food systems because of the effects on food texture. The intrinsic factors affecting water binding of food protein include amino acid composition, protein conformation, and surface polarity/hydrophobicity (Barbut, 1999; Kanu et al., 2007). The high water-binding capacity of the isolate may possibly be due to a high proportion of hydrophilic amino acids. The WAC of the FPI was higher than that of the soy protein isolate (289%) and the peanut protein isolate (135%), as reported by Kanu et al. (2007) and Wu et al. (2009), respectively. The result showed that the FPI has good WAC and could be used in several food formulations, such as meat and pastry products.

Oil absorption capacity (OAC) of the FPI and SPI was 127.48 and 134.39%, respectively (Table 1). The interactions of oil with proteins are very important in food systems because of the effects on the flavour of foods. Present finding was in line with the results reported by Kanu et al. (2007) for sesame protein isolate (129%) and soy protein isolate (134%). Gbadamosi et al. (2017) cited that the difference in OAC was related to the non-polar side chains of the protein as well as to the different conformation features of the proteins. OAC is important because oil acts as a flavour retainer and increases the mouth feel of foods (Aremu et al., 2007).

Least gelling capacity of the FPI and SPI was 3.6 and 5 %, respectively. These results agreed with Gbadamosi et al. (2017). Least gelling concentration (LGC) is the lowest protein concentration at which gel remained in the inverted tube, which is used as an index of gelation capacity. The lower the LGC, the better the gelating ability of the protein ingredient (Akintayo et al., 1999; Eltayeb et al., 2011). The ability of protein to form gels and provide a structure matrix for holding water, flavours, sugars, and food ingredients is useful in food applications and in new product development, thereby providing an added dimension to protein functionality (Oshodi et al., 1997; Eltayeb et al., 2011). The low gelation concentration observed may be an asset in the use of this isolate for the formation of curd or as an additive to other gel forming materials in food products (Aremu et al., 2007; Eltayeb et al., 2011).

Solubility

The solubility of the FPI and SPI, in the pH range of 2.0–10.0, is presented in Fig. 1. The least solubility occurred at pH 4.0 (24.54, 9.56 % for the FPI and SPI, respectively), presumably the isoelectric pH; the solubility of the FPI and SPI was 53.68 and 38.21 % at pH 7.00 and 75.35 and 50.08 % at pH 9.0, respectively. There was a rise in protein solubility below and above the isoelectric pH region. This could be attributed to the protein solubility in aqueous solutions, which is a function of pH. At pH values above and below the isoelectric pH, proteins carry a net charge; electrostatic repulsion and ionic hydration promote the solubilization of proteins. These results agreed with (Fasuan et al., 2018; Kaushik et al., 2016, Poveda et al., 2016, Onsaard, 2012, and Kanu et al., 2007). However, the maximum solubility obtained for the FPI (75.35%) is higher than that observed for the SPI (56.32%). Similar results on the solubility of the FPI were obtained by Dev and Quensel (1988), containing differing levels of mucilage.

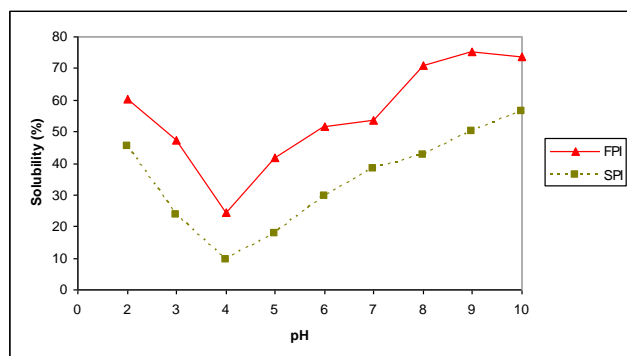


Fig. 1. The solubility of the flaxseed protein isolate (FPI) and sesame protein isolate (SPI)

Emulsifying properties

The effect of pH and the NaCl concentration on emulsion capacity (EC) of the flaxseed protein isolate (FPI) and sesame protein isolate (SPI) is shown in Fig. 2. Results revealed that the SPI and FPI had a minimum capacity (74.54 and 100.20 ml oil/g protein, respectively) at pH 4 (Fig. 2). An increase was observed on either side of pH 4. Results revealed that an alkaline pH improved the emulsion capacity more than an acidic pH. Dependence of the emulsion capacity on pH was expected, as it is known that the emulsion capacity of a total protein depends upon the hydrophilic–lipophilic balance, which is affected by pH (Sathe et al., 1982). The addition of NaCl at concentrations up to 1.0 M increased the emulsion capacity of the FPI and SPI (Fig. 2), due to the fact that

the addition of NaCl improved the solubility of the protein and, accordingly, the emulsion capacity. Beyond this salt concentration, the emulsification capacity gradually decreased due to the salting effect of NaCl. Data was in agreement with Khalid et al. (2003).

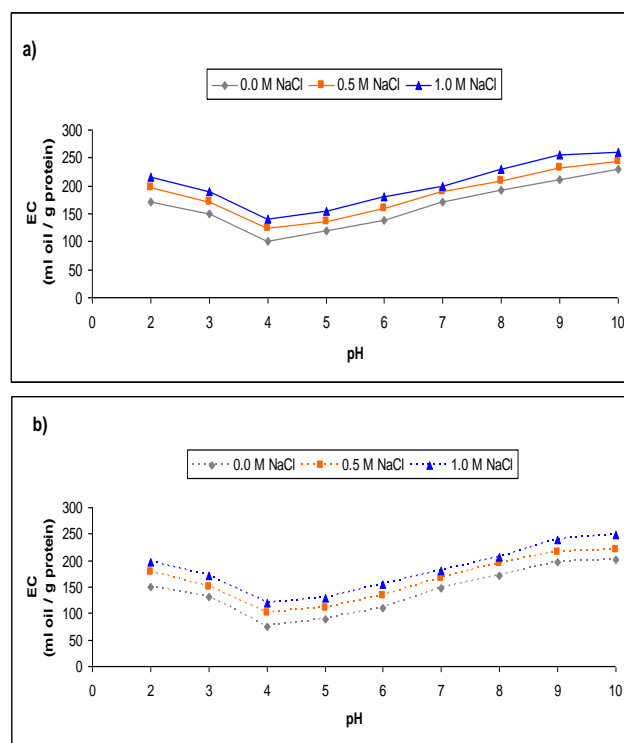


Fig. 2. The effect of pH and the NaCl concentration on the emulsion capacity of: a) flaxseed protein isolate (FPI) and b) sesame protein isolate (SPI)

The effects of pH and the NaCl concentration on the emulsion activity index (EAI) and the emulsion stability index (ESI) are shown in Figs. 3 and 4. The SPI and FPI had the lowest EAI and ESI at pH 4. The EAI of these emulsions ranged from 16.21 to 54.80 m²/g. The FPI had EAI values higher than those of the SPI. Figs. 3 and 4 illustrated an increase in the EAI and the ESI on either side of pH 4 for the SPI and FPI. However, the addition of NaCl up to (1.0 M) at pH 4 improved the EAI and ESI of the protein. This could be attributed to the fact that most proteins of plant origin can be solubilized below the pI region and above that region as well. The protein exhibited low emulsification properties when exposed to a very high pH (11–14) (Jung et al., 2005). Differences observed might account for the variations of the hydrophilic–lipophilic balance of the protein along the pH gradient from 2 to 10. Similar observations on the pH dependence of EAI have been reported (Sathe et al., 1982). The ESI values of the FPI emulsion were higher than the ESI values of the SPI emulsions (Fig. 4). The

higher the concentrations of NaCl, the higher the ESI were. The emulsion stability for the FPI obtained in this study (90%) is similar to that obtained by Kaushik et al. (2016) (86%) and Dev and Quensel (1988) (84%) for their low mucilage protein isolate.

Foaming capacity and stability

The foam capacity (FC) of the SPI and FPI (Fig. 5) was pH-dependent and was found to be lowest at pH 4 (14.25 and 17.35%, respectively).

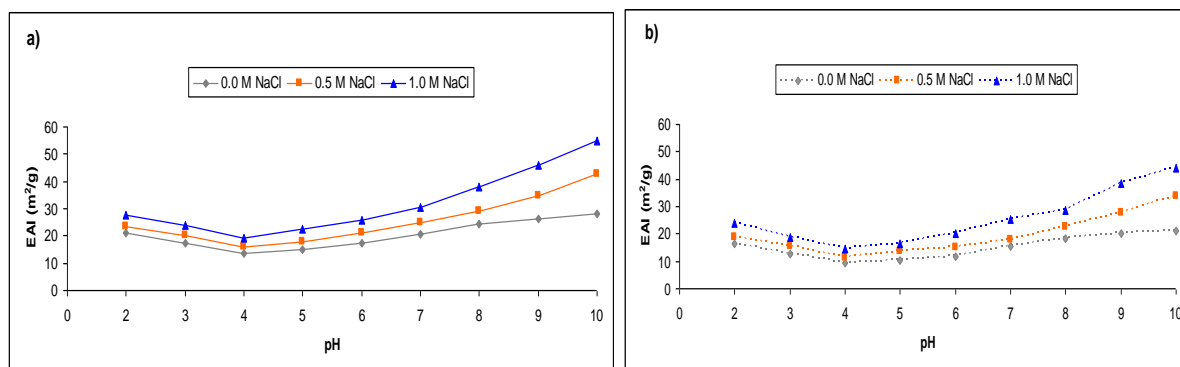


Fig. 3. The effect of pH and the NaCl concentration on the emulsion activity index (EAI) of: a) flaxseed protein isolate (FPI) and b) sesame protein isolate (SPI)

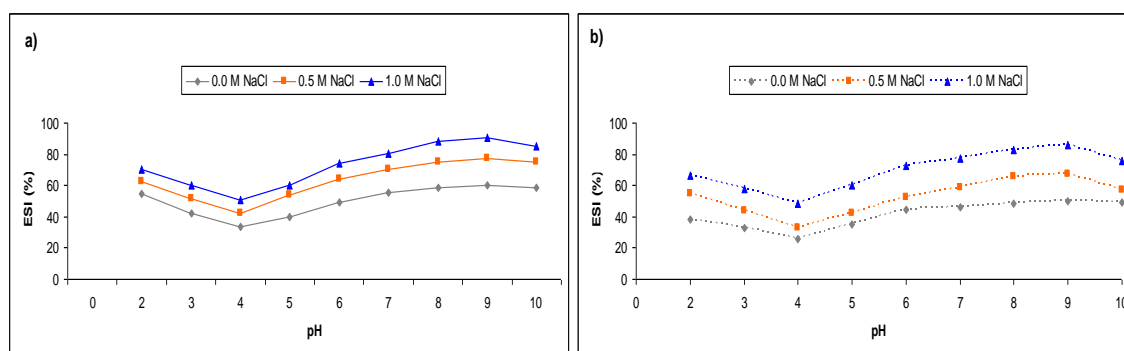


Fig. 4. The effect of pH and the NaCl concentration on the emulsion stability index (ESI) of: a) flaxseed protein isolate (FPI) and b) sesame protein isolate (SPI)

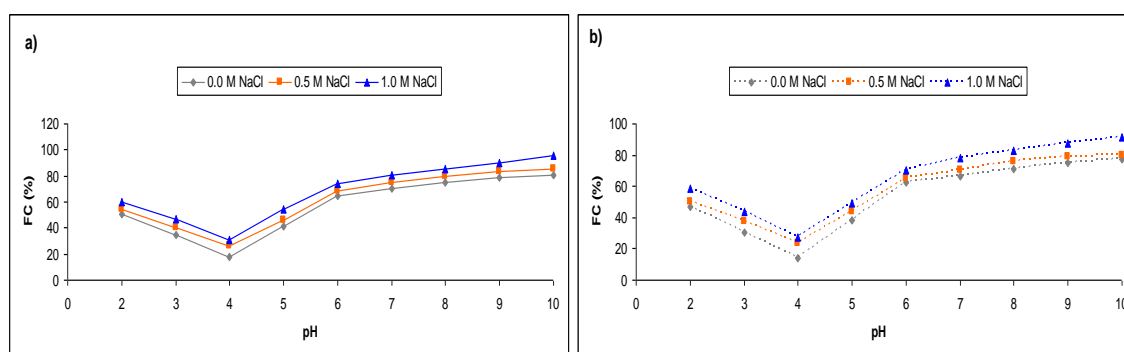


Fig. 5. The effect of pH and the NaCl concentration on the foam capacity (FC) of: a) flaxseed protein isolate (FPI) and b) sesame protein isolate (SPI)

The lowest FC was attributed to protein behaviour at its isoelectric point. Beyond pH 4, the FC was increased, especially at pH 9 and 10. The addition of

NaCl at a concentration up to 1.0 M (Fig. 5) gradually improved the FC of the protein and a higher increment was observed at this concentration.

The effects of pH and the NaCl concentration on the foam stability (FS) of the SPI and FPI are shown in Fig. 6. Data on FS showed the same tendency of FC. The addition of salt (1.0 M NaCl) has improved the FS of proteins, due to the increased solubility and surface activity of the soluble proteins. The results revealed that the foaming properties of proteins were pH-dependent.

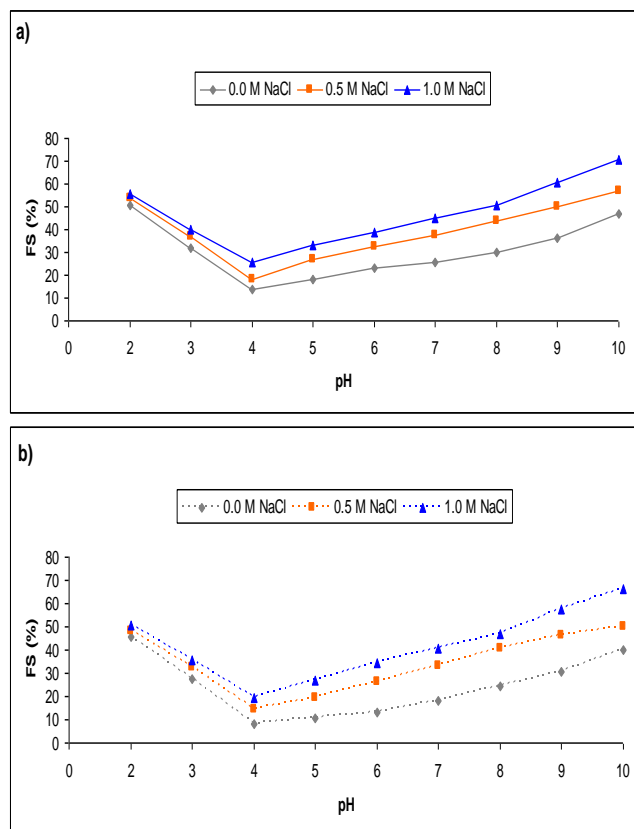


Fig. 6. The effect of pH and the NaCl concentration on the foam stability (FS) of: a) flaxseed protein isolate (FPI) and b) sesame protein isolate (SPI)

Conclusion

In conclusion, The FPI and SPI were found to be highly soluble at acidic and alkaline pH. Therefore, its emulsifying and foaming properties were high, especially with the addition of NaCl at the concentration of 1.0 M. Moreover, bulk density, water absorption and fat absorption capacities, and least gelling capacity properties were good. Therefore, the FPI and SPI can be used in food formulation systems.

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